Journal of Medical Virology 48:147–150 (1996)

Immunogenicity and Efficacy of a Killed Hepatitis A Vaccine in Day Care Center Children

Rosana Richtmann, Ricardo L. Chaves, João S. Mendonça, Selia R. Konichi, Heloisa P. Mitre, Kioko Takei, Klaus Dietz, and Bertram Flehmig

Department of Infectious Diseases (R.R., J.S.M., S.R.K., H.P.M.) and Laboratory of Clinical Pathology (K.T.), Hospital do Servidor Publico Estadual Franciso Morato de Oliveira, São Paulo, Brazil; Institute of Hygiene, Department of Medical Virology and Epidemiology of Infectious Diseases (R.L.C., B.F.) and Department of Medical Biometry (K.D.), University of Tübingen, Tübingen, Germany

The objective of this study was to characterize the immune response of children after the use of two different vaccine doses and to evaluate whether vaccination benefits children attending day care centers in areas with high anti-HAV seroprevalence. The study was conducted in a day care center with a stable population in São Paulo, Brazil. Two groups of 20 children, all seronegative for hepatitis A antibodies, were assigned randomly to receive three times 0.5 and 1.0 ml of the vaccine, the second and third dose 1 and 6 months after the first dose, respectively. There were 27 children in the control group. All children in both vaccinated groups had protective levels of antibodies in the serum after two inoculations, and serious adverse reactions were not observed.

In the eighth month of follow-up, a hepatitis A outbreak occurred in the day care center. Five children in the control group had high titers of IgM class anti-HAV, four with clinical manifestations of acute hepatitis. None of the vaccinated children developed symptoms or signs of hepatitis ($P = 0.0125$), and the estimate of vaccine efficacy was 100%. Two nonstudy children from the center also had clinical and serological evidence of acute hepatitis A. It is concluded that vaccination represents an important method for prevention of hepatitis A transmission in day care centers. The results of this pilot study justify further testing in larger groups.

KEY WORDS: HAV, GBM strain, inactivated vaccine

INTRODUCTION

Hepatitis A is a highly endemic disease in developing countries, whose prevalence closely correlates with age and socioeconomic status. In São Paulo, Brazil, the prevalence of IgG class anti-HAV (anti-HAV IgG) attains 100% among children between 2 and 11 years from a lower economic environment, 40.3% among children between 2 and 11 years from the middle class, and 90.4% among voluntary middle class adult blood donors [Panutti et al., 1985]. Worldwide, the annual incidence of hepatitis A exceeds 1.4 million cases, leading to costs of US$ 1.5–3 billion annually [Hadler, 1991]. Effective preventive measures are, therefore, clearly necessary.

Day care centers represent an important source of hepatitis A in several communities. Up to 15% of all hepatitis A cases registered in the United States acquire this infection in day care centers [Hadler, 1991]. Recently, there have been significant advances in active immunization against hepatitis A in children [Werzberger et al., 1992; Lee et al., 1993; Innis et al., 1994], but the role of immunization as a suitable method for control of transmission of this infection in day care centers has not been evaluated yet.

The purity, immunogenicity, and safety of the inactivated vaccine (HAV strain GBM) used in this study have been described previously [Heinricy et al., 1987; Flehmig et al., 1989; Garin et al., 1995]. In adults, the highest protective levels of anti-HAV antibodies were achieved with three intramuscular injections of 1.0 ml of the vaccine at 0, 1, and 6 months [Heinricy et al., 1991]. The present study attempts to characterize the immunogenicity and safety of this vaccine in healthy children and to assess whether vaccination benefits those attending day care centers in areas with high seroprevalence of anti-HAV.

MATERIALS AND METHODS

Study Design and Population

The day care center of the Hospital do Servidor Publico Estadual Francisco Morato de Oliveira in São Paulo was chosen, after the hospital's Ethics Committee gave its approval. During the full year, 180 children

Accepted for publication August 20, 1995.

Address reprint requests to Prof. Dr. Bertram Flehmig, Hygiene-Institut der Universität Tuebingen, Department of Medical Virology and Epidemiology of Infectious Diseases, Silcherstr. 7, 72076 Tübingen, Germany.

attend the center. During the last few years, outbreaks of hepatitis A occurred in this day care center.

The children who attended this center were eligible to enter the trial if they were in good health, had no previous history of hepatitis, had normal levels of serum aminotransferases, and were negative for anti-HAV IgG/IgM and hepatitis B surface antigen in serum. Parents of the children enrolled in the study, who belong to the staff of the hospital, gave written informed consent.

The children were assigned randomly to receive intramuscularly 1.0 ml, 0.5 ml at 0,1, and 6 months. The control group consisted of 27 children who received no vaccine, following the decision of their parents. Group A comprised 20 children, who received 1.0 ml of vaccine, which contains 0.3 μg of HAV protein combined with 0.5 g of aluminium hydroxide. The 20 children of group B received a 0.5 ml dose. The mean ages of groups A, B, and the controls were, respectively, 30.4 months (range, 20–44 months), 32.8 months (range, 15–57 months), and 19 months (range, 7–45 months) ($P < 0.0001$). Immediate allergic and other adverse reactions following vaccination were sought daily during the following week by the medical staff and employees. The follow-up of all children was carried out locally for 1 year.

Reporting Cases of Hepatitis A and Prescribing Passive Immunization

Clinically apparent cases of hepatitis A were detected actively in the day care center for a 1-year period after the first vaccination. Children presenting with signs or symptoms of hepatitis were examined by an investigator, and a blood sample was tested for anti-HAV IgM, serum alanine transferase, and total bilirubin. If a symptomatic child had anti-HAV IgM in serum, a blood sample for anti-HAV IgG/IgM testing was collected from all children and immune globulin (0.04 ml/kg of body weight) was administered to the control group. Passive immunization was not undertaken in vaccinees. Detection of new clinically manifested cases would continue for 2 months and the study would then be interrupted.

Clinical cases of hepatitis A were defined by anti-HAV IgM and a serum alanine transferase level higher than twice the upper limit of normal and at least two of the following signs or symptoms: icterus (associated with bilirubin level higher than 2.0 mg/dl), emesis, an axillary temperature of 37.8°C or higher, dark urine, "clay-colored" stools, abdominal pain, fatigue, or malaise. Asymptomatic cases were defined by the presence of anti-HAV IgM in serum.

Blood Testing

Anti-HAV IgM was determined in serum by the HAVAB-M assay (Abbott Laboratories). Total antibodies against HAV (anti-HAV) were detected qualitatively in serum using the HAVAB assay (Abbott Laboratories). Anti-HAV titers were determined by RIA [Flehmig et al., 1984] and results expressed in milli-international units per milliliter, in accordance with the standard reference serum of the World Health Organization. Titres >10 mIU per milliliter were considered as protective [Ambrosch et al., 1991].

Blood samples were taken from all children at the time of the first vaccination and immediately after the diagnosis of one case of hepatitis A. Additional samples were obtained from vaccinees 2 and 8 months after the first vaccination to determine the antibody response.

Statistical Analysis and Evaluation of Efficacy

The confidence interval for the vaccine efficacy was calculated according to maximum likelihood ratio test [Nam, 1995], because the standard formulas, which are based on the logarithm of the ratio of infection probabilities, are not applicable due to zero observations in the vaccinated children. The logarithms of the antibody titers were compared by the two-sample t-test.

RESULTS

Immune Response of Vaccinees

Five children, three from Group A and two from Group B, withdrew from the study before the second dose could be administered. The remaining 35 vaccinated children, who were tested for anti-HAV 4 weeks after the second dose, were found to have protecting levels of antibodies. A sharp increase in anti-HAV titers was observed in all children after the third dose, apart from one in Group A. This solitary case had the lowest anti-HAV titer in the study following full immunization, which nevertheless was more than 400-fold the minimum level of protection. The anti-HAV geometrical mean titers (GMT) in both groups after vaccination is shown in Figure 1. The antibody response of Group A (2368 mIU) was better than that of Group B (988 mIU) after the first two inoculations ($P = 0.005$). After the third inoculation, the antibody titers in Group A (19322 mIU) were also higher than that of group B (13242 mIU) ($P = 0.09$). The immunization was tolerated well, and side effects were not observed.

Hepatitis A Cases in Control Group and Efficacy of Vaccination

All 27 children in the control group were observed during the whole period of the study. Eight months after the first dose, one child from the control group presented a clinical picture of hepatitis with icterus. The child's serum was positive for anti-HAV IgM and the alanine aminotransferase levels were higher than twice the upper limit of normal. Immune globulin was administered to all nonvaccinated children 7 days after the clinical suspicion in the index case was established.

In the week following administration of immune globulin, four other cases of hepatitis A were detected in the control group, three of them with symptoms. No case of hepatitis was noted 2 or more weeks following the clinical identification of the index case. No vaccianted child had clinical manifestations of hepatitis A or anti-HAV IgM in serum.

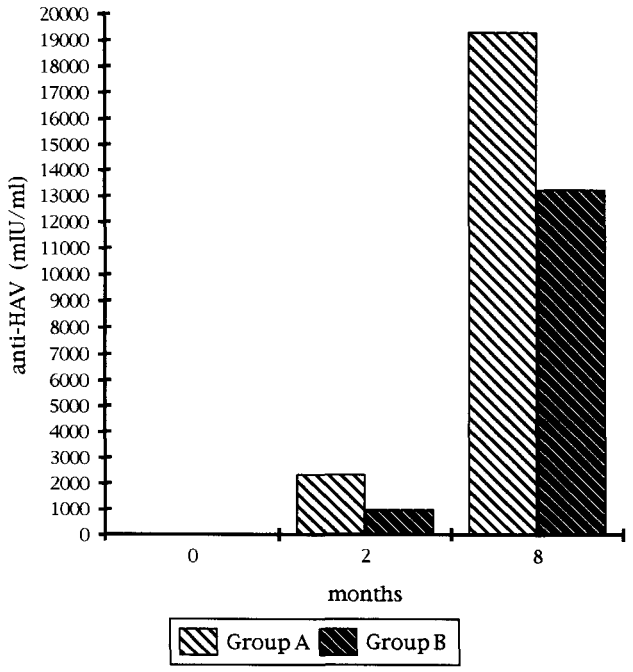

Fig. 1. Anti-HAV (GMT) response of the vaccinated children 2 and 8 months after the first vaccination. GMTs at 2 months: Group A (2368 mIU/ml); Group B (988 mIU/ml) ($P = 0.005$). GMTs at 8 months: Group A (19322 mIU/ml); Group B (13242 mIU/ml) ($P = 0.09$).

TABLE I. Estimate of Vaccine Efficacy†

Cases of hepatitis		Efficacy		
vaccinated cases/no.	nonvaccinated evaluated	estimate (%)	*P* value*	95% CI[a] (%)
0/35	5/27	100	0.0125	(44;100)

†The five vaccinees who left the study after receiving the first dose and have not developed hepatitis were not included in the analysis.
[a]Based on maximum likelihood ratio test.
*According to Fisher's exact test.

The estimate of vaccine efficacy was 100%, as summarized in Table I. The five children who received only the first dose of the vaccine also had no clinical manifestation of hepatitis.

Outbreak Impact in the Day Care Center

Two children from the day care center who were not enrolled in the study also exhibited clinical manifestations of hepatitis, with IgM anti-HAV in serum during the second week following the detection of the index case. Therefore, the total number of nonvaccinated children who developed acute hepatitis A during this outbreak increased to seven. Considering these data, the crude incidence of this outbreak in all attendees was 38.8 infections per thousand. No case of hepatitis was noted among employees or household contacts of center children.

DISCUSSION

The results show that three doses (0.5 ml or 1.0 ml) of this vaccine were well tolerated and immunogenic in children. After the first inoculation, the children who received 1.0 ml of the vaccine had significantly higher levels of anti-HAV than those who received half dose and also in comparison to adults vaccinated with the 1.0 ml dose [Heinricy et al., 1991]. The duration of vaccine-induced immunity in the children of the present study cannot be well characterized since boosters of total anti-HAV not related to vaccination occur, leading to prolongation of immunity, since re-exposure to hepatitis A virus is not uncommon in this environment.

Since no case of hepatitis A was noted 2 or more weeks after the index case, it is presumed that a common source of infection caused viral transmission to all infected children reported here. It is improbable that all hepatitis A infections detected after the index case had a short incubation period, which would mean they were secondary cases. It can, therefore, be assumed that the administration of immune globulin was successful and that all the infections detected were coprimary cases.

One may speculate that the five children who left the study after receiving just the first dose were also protected from developing disease by vaccination, because seroconversion rates >90% had been observed following a single dose of this vaccine in adults [Chaves et al., 1995]. However, the duration of immunity following a single dose has not been established as yet.

In analyzing the outbreak, two issues must be addressed. First, it is important to note that several children attending this day care center were already immune to hepatitis A, as shown during the serological screening to select the children who were eligible for this trial. Nevertheless, the crude incidence of this outbreak was 38.8 infections per thousand, indicating that the attack rate was high in comparison to outbreaks described previously in day care centers. The average attack rate of hepatitis A outbreaks in 30 day care centers, in a region with a lower anti-HAV seroprevalence (Arizona, USA) was 22 infections per thousand population [Hadler et al., 1980]. Second, it has already been demonstrated that immune globulin intervention leads to a statistically significant decrease in the extent of a day care hepatitis outbreak [Hadler et al., 1983]. These facts combined with the high proportion of symptomatic cases in the control group demonstrate the severity of the outbreak and emphasize the efficacy of the vaccine in the present study.

It is concluded that vaccination benefits children facing a high risk of HAV infection, such as those attending day care centers in areas with high anti-HAV seroprevalence. In areas with lower anti-HAV seroprevalence, where day care centers are important in the spread of hepatitis A, vaccination of attendees also could be justified and deserves evaluation.

ACKNOWLEDGMENT

R.L.C. was supported by a grant from C.A.P.E.S. (Brazilian Ministry of Education).

REFERENCES

Ambrosch F, Wiedermann G, André FE, D'Hont E, Delem A, Safary, A. (1991): Comparison of HAV antibodies induced by vaccination, passive immunization, and natural infection. In Hollinger FB, Lemon SM, Margolis HS (eds): "Viral Hepatitis and Liver Disease." Baltimore: Williams & Wilkins, pp 98–100.

Chaves RL, Pfisterer-Hunt M, Heinricy U, Dietz K, Flehmig B (1995): GBM strain hepatitis A vaccine: A 3-year follow-up. Proceedings of the International Symposium on Enterically-Transmitted Hepatitis Viruses (in press).

Flehmig B, Pfisterer M, Vallbracht A (9184): Hepatitis A in cell culture. IV. Comparison of cell-culture-produced HAV with stool derived HAV in diagnostic test systems. Medical Microbiology and Immunology 172:207–213.

Flehmig B, Heinricy U, Pfisterer M (1989): Immunogenicity of a killed hepatitis A vaccine in seronegative volunteers. Lancet 1039–1041.

Garin D, Vidor E, Wallon M, Fanget B, Brasseur P, Delome H, Caron F, Mojon M, Gravey A, Humbert Guy, Flehmig B, Peyron F (1995): Good immunogenicity of GBM strain inactivated hepatitis A vaccine in healthy male adults. Vaccine 13:220–224.

Hadler SC, Webster HM, Erben JJ, Swanson JE, Maynard JE (1980): Hepatitis A in day care centers—a community-wide assessment. New England Journal of Medicine 302:1222–1227.

Hadler SC, Erben JJ, Mathews D, Starko K, Francis DP, Maynard JE (1983): Effect of immunoglobulin on hepatitis A in day-care centers. Journal of the American Medical Association 249:48–53.

Hadler SC (1991): Global impact of hepatitis A virus infection changing patterns. In Hollinger FB, Lemon SM, Margolis HS (eds): "Viral Hepatitis and Liver Disease." Baltimore: Williams & Wilkins, pp 14–20.

Heinricy U, Stierhof YD, Pfisterer M, Flehmig B (1987): Properties of a hepatitis A virus candidate vaccine strain. Journal of General Virology 68:2487–2493.

Heinricy U, Pfisterer M, Stierhof YD, Flehmig B (1991): Schedule-dependent immune response to hepatitis A vaccination. In Hollinger FB, Lemon SM, Margolis HS (eds): "Viral Hepatitis and Liver Disease." Baltimore: Williams and Wilkins, pp 108–110.

Innis BL, Snitbhan R, Kunasol P, Laorakpopngse T, Poopatanakool W, Kozik CA, Suntayakorn S, Suknuntapong T, Safary A, Tang DB, Boslego JW (1994): Protection against hepatitis A by an inactivated vaccine. Journal of the American Medical Association 271: 1328–1334.

Lee S, Lo K, Chan C, Yu M, Wang Y, Safary A (1993): Immunogenicity of inactivated hepatitis A vaccine in children. Gastroenterology 104:1129–1132.

Nam JM (1995): Confidence limits for the ratio of two binominal proportions based on likelihood scores: Non-iterative method. Biometric Journal 37:375–379.

Pannuti CS, Mendonca JS, Carvalho MJM, Oselka GW, Amato Neto V (1985): Hepatitis A antibodies in two socioeconomically distinct populations of São Paulo, Brazil. Revista do Instituto de Medicina Tropical de São Paulo 27:162–164.

Werzberger A, Mensch B, Kuter B, Brown L, Lewis J, Sitrin R, Miller W, Shouval D, Wiens B, Calandra G, Ryan J, Provost P, Nalin D (1992): A controlled trial of a formalin-inactivated hepatitis A vaccine in healthy children. New England Journal of Medicine 327:453–457.